

CLAIMS:

1. A method of assaying pyrrole-containing biological compounds comprising:

- i) contacting a biological compound with one of:
  - a) an optionally labelled derivatizing agent (bound to or able to bind with a solid support), wherein the derivatizing agent forms a reaction product with the biological compound, followed by exposure to a detectable molecule which forms a complex with the reaction product; or
  - b) an optionally labelled derivatizing agent not bound to a solid support, wherein the derivatizing agent forms a reaction product with the biological compound, followed by exposure to a binding agent specific to the biological compound in the reaction product, said binding agent being bound to a solid support; or
  - c) a binding agent bound to a solid support, said binding agent being specific to the biological compound and forming a complex therewith, followed by exposure to an optionally labelled, derivatizing agent which

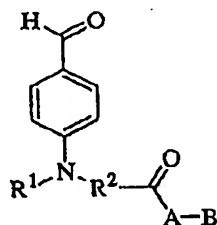
forms a reaction product with the biological compound moiety of said complex,

and

- ii) determining the amount of bound biological compound by detecting the detectable molecule, or by determining the amount of free or bound binding agent or by measuring the amount of label present; thereby assaying pyrrole containing biological compounds.

2. The method according to Claim 1 comprising:

- (a) contacting the biological compound with a derivatizing agent of the following structure in the bound form:



wherein  $R^1$  is an alkyl group,  $R^2$  is an alkyl group, A is a linking group and B is a solid support, and wherein the contact induces formation of a reaction product, and wherein the reaction product comprises the covalent attachment of the biological compound to the derivatizing agent;

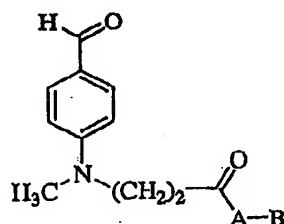
- (b) contacting the reaction product with a

detectable molecule, wherein the contact induces specific binding of the detectable molecule to the reaction product to provide a complex;

- (c) detecting the detectable molecule; thereby assaying pyrrole containing biological compounds.

3. The method according to Claim 2, wherein  $R^1$  is a straight-chain alkyl group containing 1 to 10 carbon atoms, and  $R^2$  is a straight-chain alkyl group containing 1 to 10 carbon atoms.
4. The method according to Claim 2, wherein A is a heteroalkyl group.
5. The method according to Claim 2, wherein the detectable molecule is a first monoclonal antibody.
6. The method according to Claim 3, wherein  $R^1$  is a straight-chain alkyl group containing 1 to 5 carbon atoms and  $R^2$  is a straight chain alkyl group containing 1 to 5 carbon atoms.
7. The method according to Claim 4, wherein A is a heteroalkyl group comprising at least one nitrogen atom.

8. The method according to Claim 5, wherein the method further comprises contacting the complex with a second monoclonal antibody that specifically binds to the first monoclonal antibody.
9. The method according to Claim 6, wherein the derivatizing agent is of the following structure in bound form:



10. The method according to Claim 9, wherein A is a heteroalkyl group containing at least one nitrogen atom, and wherein the detectable molecule is a first monoclonal antibody.
11. The method according to Claim 10, wherein the method further comprises contacting the complex with a second monoclonal antibody that specifically binds to the first monoclonal antibody.
12. The method according to Claim 1 comprising:
- contacting the biological compound with an optionally labelled derivatizing agent in solution to form a reaction product

therewith, followed by exposure to a binding agent bound to a solid support wherein the binding agent is specific to the biological compound in the reaction product; and

- b) determining the amount of bound biological compound by determining the amount of labelled derivatizing agent bound to the solid support; thereby assaying the pyrrole-containing compound.

- 13. The method according to Claim 12 wherein the derivatizing agent is biotinylated Ehrlich's reagent.
- 14. The method according to Claim 12 wherein the derivatizing agent is labelled with a radio-label, fluorescent label or an enzyme label.
- 15. The method according to Claim 12 wherein the solution containing the reaction product is neutralised prior to contact with the binding agent.
- 16. The method according to Claim 1 comprising;
  - a) contacting the biological compound with a derivatizing agent in solution to form a reaction product, wherein the derivatizing

agent comprises a first partner of a strong binding pair;

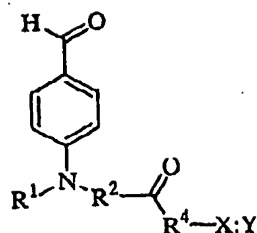
- b) contacting the reaction product with a solid support having a second partner of the strong binding pair on its surface, to form a bound complex with the reaction product;
- c) contacting the bound complex with a detectable molecule;
- d) determining the amount of bound biological compound;  
thereby assaying the pyrrole-containing biological compound.

17. The method according to Claim 16 wherein the solution containing the reaction product is neutralised prior to contact with the solid support.

18. The method according to Claim 16 wherein the detectable molecule is a monoclonal antibody specific to the biological compound and the amount of bound biological compound is determined by detecting the amount MAb bound to the solid support.

19. A method as claimed in Claim 16 comprising:

- a) contacting the biological compound with a derivatizing agent of the following structure in the bound form;



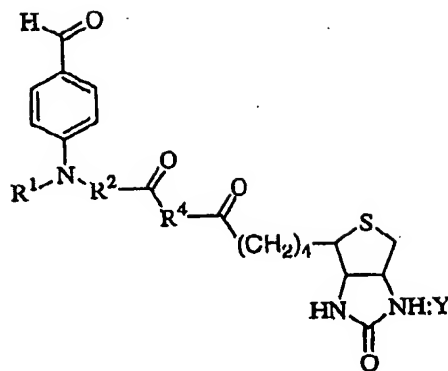
wherein R<sup>1</sup> is an alkyl group, R<sup>2</sup> is an alkyl group, R<sup>4</sup> is a heteroalkyl group, X is the first labelling molecule and acts as a first partner of a strong binding pair and Y is the second labelling molecule and acts as a second partner of a strong binding pair on the surface of a solid support, and wherein the contact induces formation of a reaction product, and wherein the reaction product comprises the covalent attachment of the biological compound to the derivatizing agent;

- b) contacting the reaction product with a detectable molecule, wherein the contact induces specific binding of the detectable molecule to the reaction product to provide a complex;
- c) detecting the detectable molecule; thereby assaying pyrrole containing biological compounds.

20. The method according to Claim 19, wherein R<sup>1</sup> is a straight-chain alkyl group containing 1 to 10

carbon atoms, and  $R^2$  is a straight-chain alkyl group containing 1 to 10 carbon atoms.

21. The method according to Claim 19, wherein  $R^4$  is a straight-chain heteroalkyl group containing 2 to 10 carbon atoms and at least 2 heteroatoms.
22. The method according to Claim 19, wherein Y is a solid support having avidin on its surface.
23. The method according to Claim 19, wherein the detectable molecule is a first monoclonal antibody.
24. The method according to Claim 19, wherein  $R^1$  is a straight-chain alkyl group containing 1 to 5 carbon atoms, and  $R^2$  is a straight chain alkyl group containing 1 to 5 carbon atoms.
25. The method according to Claim 18, wherein the method further comprises contacting the complex with a second monoclonal antibody that specifically binds to the first monoclonal antibody.
26. The method according to Claim 25, wherein the derivatizing agent, in bound form, is of the following structure:



wherein  $R^1$  is a straight-chain alkyl group containing 1 to 5 carbon atoms,  $R^2$  is a straight-chain alkyl group containing 1 to 5 carbon atoms,  $R^4$  is a straight-chain heteroalkyl group containing 2 to 10 carbon atoms and at least 2 heteroatoms, and Y is a solid support having avidin or streptavidin on its surface.

27. The method according to Claim 26, wherein  $R^1$  is  $-\text{CH}_3$ ,  $R^2$  is  $-\text{CH}_2\text{CH}_2-$  and  $R^4$  is  $-\text{NH}(\text{CH}_2)_5\text{NH}-$  or  $-\text{NH}(\text{CH}_2)_2\text{SS}(\text{CH}_2)_2\text{NHC}(\text{O})(\text{CH}_2)_5\text{NH}-$ .
28. The method according to Claim 27, wherein  $R^4$  is  $-\text{NH}(\text{CH}_2)_5\text{NH}-$ .